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Original Research

Antibacterial effectiveness of combined sodium hypochlorite and diode laser therapy with different parameters against S. Aureus biofilm

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Abstract
Aim: In this study, we aimed to assess the antibacterial efficacy of both single and combined sodium hypochlorite (NaOCl) and diode laser on Staphylococcus aureus (S. aureus) biofilm.
Material and Methods: Eighty-four premolars were randomly assigned to groups. The Experimental groups were as follows: group 1; 5.25% NaOCl; groups 2-4; diode laser (0,7; 1,5; 2,5 W); Groups 5-7; combined laser and NaOCl. Statistical evaluations were made by Kruskal -Wallis and Mann- Whitney U tests.
Results: The 2.5 W diode laser showed a high potential for elimination of S.aureus, similar to NaOCl ($p>0.05$). 0,7 W and 1,5 W diode lasers showed less antibacterial activity than the other groups ($p<0.05$). There was no difference between single and combined applications ($p>0.05$).
Discussion: Diode laser application did not show a synergistic effect with NaOCl. The 2,5 W laser parameter was also found to be sufficient on its own. The effectiveness of laser irrigation has been shown to depend on parameters.

Keywords
Biofilm, Diode Laser, Sodium Hypochlorite, Staphylococcus Aureus

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Introduction

Biofilm is a complex polymicrobial community that can contain different types of microorganisms and can adhere to surfaces [1,2]. Root canal irrigation aims to achieve the goal of destroying intracanal biofilms as well as killing bacteria [3,4]. Sodium hypochlorite (NaOCl) is considered the most effective solution of for the root canal irrigation solutions [5,6]. Apart from In addition to having side effects such as concentration-dependent efficacy depending on concentration and toxic effect on for tissues in the periapical region, it cannot sufficiently penetrate all microorganisms in the canal [7,8]. In recent years, laser irradiation hasve begun to be used in endodontic treatment [9]. It has been reported that the performance of root canal treatment increases when used for disinfection in endodontic practice [10,11]. Diode laser application is expected to have a higher penetration depth and bactericidal effect compared to the limited penetration behavior of chemical disinfectants [12]. In the literature, there are limited studies on the comparison of the combined use of endodontic irrigation methods in terms of antibacterial properties [13-15]. Therefore, an understanding of the interactions between irrigation applications will be will be helpful for optimal treatment in root canal treatment cases [16]. Theis current study aimeds to evaluate the antibacterial activity of both single and combined use of NaOCl and diode laser (0.7; 1.5; 2.5 W) treatments on S. aureus biofilm in vitro. The null hypothesis is that there is no difference between the groups.

Material and Methods

This study was conducted between February and April 2021 atin Kafkas University Medical Faculty Hospital of Kars, Turkey. The study received Ethical approval with a number of No. 80576354-050-99/51 was obtained for the study from the Ethics Committee of Kafkas University.

Preparation of tooth specimens

The sample of the study consisted of 84 human teeth that were extracted for periodontal or orthodontic reasons, single root, single canal, and untreated endodontically. The teeth wereas cleaned with a hand curette and the root length was fixed at 14 mm. The root canals of the teeth were prepared with X1-X3 rotary ProTaper NEXT and sterilized using 5.25% NaOCl, 17% EDTA, saline solution, 5 ml each. The apical foramen of each root was covered with composite resin, and the outer surface of the root was covered with 2 layers of nail polish. The samples were placed in 2 ml tubes and sterilized in an autoclave at 121°C for 15 minutes.

Biofilm Formation

S. aureus ATCC 25923 strain was passaged into Tryptic Soy Agar (TSA, Oxoid) to obtain fresh culture and incubated overnight incubation in Tryptic Soy Broth (TSB, Oxoid, Milan, Italy). McFarland turbidity was adjusted with a crystalspecTM instrument. The McFarland standard number 0.5 was used to cure blood agar plates to achieve 1.5 X 108 colony-forming units (CFU/ml) of bacterial growth. A 1/100 ratio of fresh culture was added into TSB with 2% D-glucose, and 1 ml was taken and added to the tooth. Incubation was carried out at 37°C under aerobic conditions for 48 hours.

Preparation of experimental and control groups

The Samples were divided into 7 experimental groups and positive (n:10, steril salin)-negative (n:4, no preparation) control groups. The Experimental groups were as follows:

Group 1 (n:10); The irrigation procedure of the samples was carried out with 5 ml of 5.25% NaOCl for 1 minute.

Groups 2-4 (n:10); Diode laser (Epic, Biolase Tech., CA, USA) with a wavelength of 940 nm was applied in continuous mode (CW) with the help of an endodontic tip (ezTip Endo, 200 µm, 14 mm). Laser irradiation was transmitted from the apical of the root to the coronal in circular motions, that is, 3 times 20 s application period (ap) and 15 s waiting period (wp). Fibers were sterilized for each tooth between irradiations.

Groups 5-7 (n:10); Laser irradiation was applied directly to the remaining solution after irrigation with 5% NaOCl, as in groups 2-4.

The final irrigation was carried out with sterile saline for all groups.

Bacterial evaluation

Samples were taken from the root canals of the teeth subjected to bacterial inoculation before and after the disinfection process. The collection of samples was carried out by scraping the all surfaces with a sterile #25 K-File (Dentsply, Maillefer) for 20 s and then keeping the paper cone placed in the canal for 30 seconds. Then, the samples were placed in microcentrifuge tubes containing 1 ml of PBS solution and sonicated for 5 minutes followed by vortexing for 2 minutes. Then, bacterial counts were performed.

Statistic Evaluation

Statistical analysis was performed using IBM SPSS Statistics 18 (IBM SPSS, Turkey). Kruskal- Wallis and Mann- Whitney U and Wilcoxon sign tests were used. The $p< 0.05$ level was consideredaccepted significance.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

When analyzed Through the analysis before and after the root canal disinfection treatments, a significant decrease was found in the number of CFUs in all groups ($p< 0.05$) (Table 1) (Figure 1). The decrease in the number of CFUs was more significant in all disinfection protocols when compared to the control group.

Pair and multiple comparisons of the groups are shown in the Table 2. A significant difference was found in comparisons between groups (p= 0,020). The percentage decrease rate in groups 2 and 3 was found to be significantly lower than in

Table 1. Counts of S. Aureus Before And After Disinfection Protocols

Groups	Before irrigation			After irrigation		
	Mean	Median	Range	Mean	Median	Range
1	2,6529E7	2,6000E7	48400000,00	,0000	,0000	,0000
2	3,8486E7	3,7000E7	76600000,00	335,714,286	60,000,000	200000,00
3	2,5217E7	2,2600E7	76600000,00	316,666,667	310,000,000	57000,00
4	8,9667E6	6,0000E6	24200000,00	3,333,333	,0000	1000,00
5	1,7420E7	1,4400E7	28500000,00	9,000,000	,0000	9000,00
6	2,2360E7	2,1000E7	33400000,00	14,000,000	,0000	4000,00
7	1,5033E7	1,2500E7	32600000,00	,0000	,0000	,0000

Table 2. Statistical Comparisons Among All Groups

Groups	2	3	4	5	6	7
1	,009*	,001*	,112	,403	,081	1,00
2		,113	,131	,016*	,445	,014*
3			,003*	,000*	,021*	,002*
4				,300	,526	,140
5					,137	,439
6						,104

*p<0.05

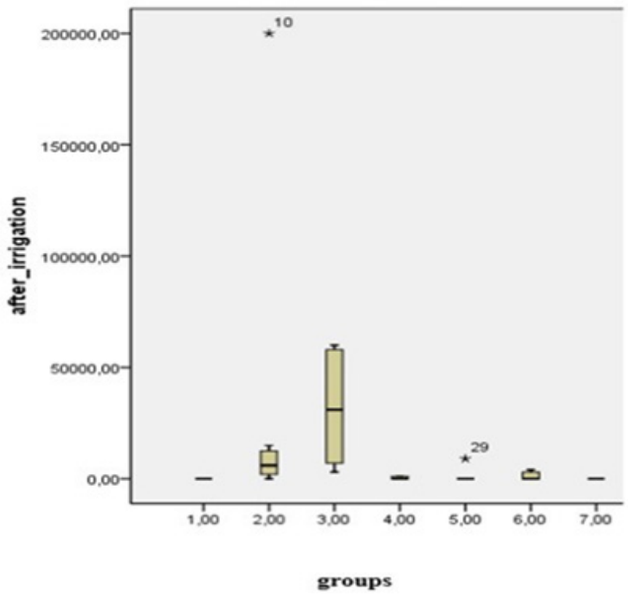

Figure 1. Bacterial Counting After Irrigation Protocols

Group 1 (p= 0,009; p= 0,001) ; no significant difference was found among the other groups. No significant difference was found between groups 2 and 3 (p= 0,113).

Discussion

Successful root canal treatment depends on the complete removal of intracanal microorganisms [10]. The aim of this study is to assess and compare the synergistic effect of different disinfection methods and irrigation agents on S. aureus biofilm. In this study, the null hypothesis was rejected.

The advantages of lasers over conventional methods in endodontic treatment have been tried to be investigated [9-11]. Many different wavelengths and settings were used in the studies [11,12]. This study was performed with a 940 nm diode laser in continuous mode (CW). In the current study, we also used a laser in combination with NaOCl. This study observed that after 48 hours, all experimental protocols decreased the bacterial load, while groups 2 and 3, which were only the laser group, achieved less disinfection.

Diode laser irradiation was reported to contribute to a significant reduction of bacteria in primary teeth to disinfect the root canal in an in vivo study [13]. An another in vivo study showed that 2% chlorhexidine, 1% NaOCl and diode laser irradiation (4x5s ap; 20s wp) were successful in reducing root canal infection, but there was no significant difference between them [14]. In the current study, the laser irradiation protocol was 3x20 s ap and 15 s wp. Studies have similarly reported diode laser irradiation as a possible complement to existing protocols for disinfection of the root canal system.

When Kushwah et al. [15] compared the protocols of with 3% NaOCl, ozonated water, diode laser (3 W; 4x5 s ap, 10 wp), their bacterial activities were found to be similar to our study. One another study showed that the application of laser (4x5 s ap) alone did not have sufficient antimicrobial activity [16]. On the contrary, in our study, a 2.5 W laser was found to have similar antibacterial effects as NaOCl. The difference between the two studies may be due to application times and power.

The study concluded that the 3W diode laser in continuous mode was more effective than pulse mode and 5.25% NaOCl [17]. In the current study, only the continuous mode of the laser was used. In another study, 2.5% NaOCl, diode laser (pulse mode; 2.5 W), and the combination of laser with NaOCl were reported to have similar antibacterial activity [18]. In our study, continuous mode was used at 2.5 W power and the results were similar.

In this study, current observations indicate that irrigation with NaOCl and 2.5 W diode laser irradiation is effective on Staphylococcus aureus. Further studies are needed to evaluate the disinfection effectiveness of different parameters of the diode laser alone in root canal treatment from different perspectives.

Limitations of the study

Considering that there are many bacterial species responsible for root canal infections, a single bacterial species was examined in this study. Only the effectiveness of the continuous mode of the diode laser has been investigated.

Conclusion

The potential application of the diode laser in 0.7 W and 1.5 W applications should not replace conventional treatment regimens, but should be seen as a possible adjunct. The microbial efficiency of 2.5 W diode laser application is similar to 5.25% NaOCl.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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